



EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to isolated soy protein and reduction of blood LDL-cholesterol concentrations pursuant to Article 14 of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to isolated soy protein and reduction of blood LDL-cholesterol concentrations pursuant to Article 14 of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from the European Natural Soyfood Manufacturers Association (ENSA), the European Vegetable Protein Federation (EUVEPRO) and the Soya Protein Association (SPA), submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Belgium, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to isolated soy protein (ISP) and reduction of blood LDL-cholesterol concentrations, referring to disease risk reduction. The food constituent that is the subject of the health claim, ISP (as defined by the applicant), is sufficiently characterised in relation to the claimed effect. A reduction of blood LDL-cholesterol concentrations is a beneficial physiological effect. A reduction in blood LDL-cholesterol concentrations reduces the risk of CHD. In weighing the evidence, the Panel took into account that under similar conditions four randomised controlled trials (RCTs) reported an effect of ISP on blood LDL/non-HDL cholesterol concentrations, whereas 14 RCTs did not report such an effect, and another RCT showed no consistent effects. The Panel also took into account that most of these RCTs were at high risk of bias, that differences in the results obtained between trials appear unrelated to the dose of ISP used, to sample size or to study duration, and that the evidence provided in support of a possible mechanism was not convincing. A cause and effect relationship has not been established between the consumption of ISP (as defined by the applicant) and a reduction in blood LDL-cholesterol concentrations.

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KEY WORDS

Isolated soy protein, ISP, blood cholesterol, LDL-cholesterol, health claims.

¹ On request from the Competent Authority of Belgium following an application by ENSA/EUVEPRO/SPA, Question No EFSA-Q-2011-00784, adopted on 18 January 2012.

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SUMMARY

Following an application from the European Natural Soyfood Manufacturers Association (ENSA), the European Vegetable Protein Federation (EUVEPRO) and the Soya Protein Association (SPA), submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Belgium, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to isolated soy protein (ISP) and reduction of blood LDL-cholesterol concentrations.

The scope of the application was proposed to fall under a health claim referring to disease risk reduction.

The food constituent that is the subject of the health claim is “protein-rich soybean component with limited quantities of macro- and micronutrients”, which upon a request from EFSA was further defined by the applicant as ISP. The Panel considers that ISP (as defined by the applicant) is sufficiently characterised in relation to the claimed effect.

The claimed effect is “reduction of total and LDL-cholesterol in healthy subjects with normal or mildly elevated blood cholesterol; a reduction of total and LDL cholesterol has been shown to reduce the risk of heart disease”. The target population proposed by the applicant is healthy subjects with normal or mildly elevated blood cholesterol levels. The Panel considers that reduction of blood LDL-cholesterol concentrations is a beneficial physiological effect. A reduction in blood LDL-cholesterol concentrations reduces the risk of CHD.

The applicant identified 23 RCTs, which used ISP and one RCT which used the water insoluble fraction of a partially hydrolysed soy protein as interventions for the scientific substantiation of the claim, and presented an unpublished meta-analysis of 23 of these 24 RCTs. In addition, six RCTs, which used whole soy foods, and eight observational studies were provided as well as one animal and two *in vitro* studies on the mechanism by which ISP could exert the claimed effect. Eleven published meta-analyses and one unpublished systematic review were presented in support of the claim.

The Panel considers that the study which assessed the effect of the water insoluble fraction of a partially hydrolysed soy protein on blood cholesterol concentrations cannot be used to substantiate a claim on ISP owing to its compositional differences, which might have had an impact on the claimed effect. Four of the 23 RCTs on ISP provided did not allow conclusions to be drawn on the effects of ISP on blood cholesterol concentrations owing to inadequate methodology or insufficient reporting.

Fourteen of the remaining 19 RCTs had a parallel design while five had a cross-over design. All subjects (n=1,947) who entered data analysis had normal or moderately elevated blood LDL-cholesterol concentrations at baseline.

The Panel notes that four RCTs with 15-41 subjects per group reported a statistically significant effect of ISP on blood LDL/non-HDL cholesterol concentrations at doses of 20 to 40 g per day consumed for six to 24 weeks. However, the Panel also notes that 14 RCTs with >15 subjects per group/period (four of which had about 90 subjects per group/period), did not report such an effect under similar conditions (20 to 40 g ISP per day given for at least six weeks in all but four studies), and that the effects found in one RCT were not consistent. In addition, most of the RCTs were at high risk of bias, and differences in the results obtained between trials appeared to be unrelated to the dose of ISP used, sample size or study duration.

The applicant also provided a meta-analysis performed on 23 of the 24 RCTs presented as the main body of evidence for the scientific substantiation of the claim. The Panel notes that this meta-analysis did not include any additional studies to those provided individually by the applicant for the scientific substantiation of the claim. The Panel also notes that this meta-analysis includes the four RCTs identified above as having inadequate methodology or insufficient reporting, as well as the one RCT

which used the water insoluble fraction of a partially hydrolysed soy protein as intervention, and considers that this meta-analysis does not provide any additional information to the individual studies considered for the scientific substantiation of the claim.

Ten of the 11 published meta-analyses as well as the unpublished systematic review provided were not designed to assess the effects of ISP but rather of isoflavones or of soy protein from different sources, including soy foods, on blood cholesterol concentrations. Although results of (sub-group) analyses for ISP were presented in some of the meta-analyses, all of these contained studies not considered pertinent to the claim by the applicant, or the reporting in the publication was insufficient for a full scientific evaluation. Also, the meta-analysis, which was designed to assess the effect of ISP on blood lipid concentrations, included studies which were considered by the applicant as not pertinent to the claim owing to methodological limitations in the design, to the high doses of ISP used, or to the characteristics of the study population. The Panel considers that no conclusions can be drawn from these meta-analyses and the systematic review for the scientific substantiation of the claim.

The six RCTs and the eight epidemiological studies on whole soy foods, which were provided by the applicant as supportive evidence for the scientific substantiation of the claim, did not allow conclusions to be drawn on the scientific substantiation of the claim on ISP and blood cholesterol concentrations, owing to the differences in macronutrient and fibre composition between whole soy foods and ISP, which might have had an impact on the claimed effect.

With regard to a possible mechanism by which ISP could exert the claimed effect, the applicant suggests that peptides derived from intestinal digestion of ISP can enter the circulation and exert a direct effect on the hepatic metabolism of cholesterol by increasing the expression of the hepatic LDL receptor. As evidence for this mechanism, the applicant provided two *in vitro* studies on the effect of the soybean 7S globulin α' subunit and a purified recombinant polypeptide containing the N-terminal extension region of the soybean α' subunit on LDL-receptor mediated LDL uptake and degradation in a human hepatoma cell line (Hep G2). The applicant also presented one animal study in male Sprague-Dawley rats, which assessed the effect of soybean 7S globulin and soybean 7S globulin α' subunit on blood cholesterol concentrations and β -VLDL receptor activity. The Panel considers that results from rat studies cannot be extrapolated to humans because of differences in lipid metabolism between these two species, that the evidence provided did not establish that peptides derived from the intestinal digestion of ISP can be absorbed intact and that the evidence provided in the *in vitro* studies is not sufficient to predict an effect of peptides derived from the soybean 7S globulin α' subunit on the LDL-receptor mediated LDL uptake and degradation in humans.

In weighing the evidence, the Panel took into account that, under similar conditions four RCTs reported an effect of ISP on blood LDL/non-HDL cholesterol concentrations, whereas 14 RCTs did not report such an effect and another RCT showed no consistent effects. The Panel also took into account that most of these RCTs were at high risk of bias, that differences in the results obtained between trials appear unrelated to the dose of ISP used, sample size or study duration and that the evidence provided in support of a possible mechanism was not convincing.

The Panel concludes that a cause and effect relationship has not been established between the consumption of ISP (as defined by the applicant) and a reduction in blood LDL-cholesterol concentrations.

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BACKGROUND

Regulation (EC) No 1924/2006⁴ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14 to 17 of this Regulation lay down provisions for the authorisation and subsequent inclusion of reduction of disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

According to Article 15 of this Regulation, an application for authorisation shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 10/06/2011.
- The scope of the application was proposed to fall under a health claim referring to disease risk reduction.
- The scientific evaluation procedure started on 30/06/2011.
- On 14/10/2011, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application, and the clock was stopped on 18/10/2011 in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
- On 17/11/2011, EFSA received the requested information as submitted by the applicant and the clock was restarted.
- During its meeting on 18/01/2012, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to isolated soy protein and reduction of blood LDL-cholesterol concentrations.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16 of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: isolated soy protein and reduction of blood LDL-cholesterol concentrations.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of isolated soy protein, a positive assessment of its safety, nor a decision on whether isolated soy protein is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 17 of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: European Natural Soyfood Manufacturers Association (ENSA), Neo Building Box 7, Rue Montoyer 51, 1000 Brussels, Belgium; European Vegetable Protein Federation (EUVEPRO), c/o AGEF, Boulevard Saint-Michel 77-79, B-1040 Brussels, Belgium; Soya Protein Association (SPA), Food and Drink Federation, 6 Catherine Street, London WC2B 5JJ, United Kingdom.

Food/constituent as stated by the applicant

According to the applicant, the food, which is the subject of the claim, is “protein-rich component from soybeans with limited quantities of other macronutrients and micronutrients”, which, upon a request from EFSA, has been further defined by the applicant as isolated soy protein.

Health relationship as claimed by the applicant

According to the applicant, the claimed effect relates to the reduction of total and LDL cholesterol in healthy subjects with normal or mildly elevated blood cholesterol. Reduction of total and LDL cholesterol has been shown to reduce the risk of heart disease.

Wording of the health claim as proposed by the applicant

The following wording is proposed by the applicant: Protein-rich soybean component has been shown to lower/reduce blood cholesterol; blood cholesterol lowering may reduce the risk of (coronary) heart disease.

Specific conditions of use as proposed by the applicant

The applicant proposes that the claim can be made if the serving of food/beverage:

1. Includes a statement:

(a) Identifying what constitutes a serving and the amount of soy protein provided in each serving expressed as grams or millilitres, e.g. “One 200 mL glass of soymilk contains 6.5 g soy protein”, “One 125 g pot of soy dessert contains 3.8 g soy protein”, etc.

(b) Identifying that the consumption of protein-rich soybean component equivalent to at least 12 g soy protein/day has been shown to reduce blood cholesterol.

2. Provide(s) a “reasonable amount” of protein-rich soybean component; this is defined as 15 % of the target daily amount. The evidence demonstrates that protein-rich soy bean component equivalent to 12-40 g soy protein is effective in reducing blood cholesterol and therefore the requirement per serving is prescribed as >3.75 g soy protein.

3. Meets the requirement for a source of protein claim: “a claim that a food is a source of protein, and any claim likely to have the same meaning for the consumer, may only be made where at least 12 % of the energy value of the food is provided by protein”.

4. Meets the requirement that protein-rich soybean component contains naturally occurring isoflavones (typically 0.7-6.3 mg/g protein).

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is “protein-rich soybean component with limited quantities of macro- and micronutrients”, which, upon a request from EFSA, has been further defined by the applicant as isolated soy protein (ISP).

The CODEX General Standard for Soy Protein Products (CODEX STAN 175-1989) (Codex Alimentarius, 1989) defines ISP as the component that is left by the reduction or removal, under Good Manufacturing Practices, from soybeans of certain of the major non-protein constituents (water, oil, carbohydrates) in a manner to achieve a largely protein component, which contains ≥ 90 % protein, ≤ 0.5 % crude fibre, ≤ 8 % ash and residual oils, which usually range between 0.5 and 1 % (Liu, 1997). ISP also contains isoflavones, which, according to the applicant, usually vary between 0.7 and 6.3 mg isoflavones per g protein.

Protein can be measured in foods by established methods.

The Panel considers that the food constituent, ISP (as defined by the applicant), which is the subject of the health claim, is sufficiently characterised in relation to the claimed effect.

2. Relevance of the claimed effect to human health

The claimed effect is “reduction of total and LDL-cholesterol in healthy subjects with normal or mildly elevated blood cholesterol; a reduction of total and LDL cholesterol has been shown to reduce the risk of heart disease”. The target population proposed by the applicant is healthy subjects with normal or mildly elevated blood cholesterol levels.

Coronary heart disease (CHD) is a leading cause of mortality and morbidity in European populations with over 1.9 million deaths in the European Union and over 4.35 million deaths in Europe each year (Petersen et al., 2005). Elevated blood cholesterol is an important modifiable risk factor in the development of CHD (WHO, 2002).

It has been shown that blood cholesterol can be decreased by drugs, and by dietary and lifestyle changes (Denke, 2005; Gordon, 2000; Katan et al., 2003; Law, 2000; Ornish et al., 1998; Pedersen et al., 2005; Van Horn et al., 2008).

The Panel considers that reduction of blood LDL-cholesterol concentrations is a beneficial physiological effect. A reduction in blood LDL-cholesterol concentrations reduces the risk of CHD.

3. Scientific substantiation of the claimed effect

Harland and Haffner (2008) performed a literature search (January 1995 to September 2007) in Medline, Embase, SciSearch and Current Contents to identify human studies using the search terms “soy(a)” and “cholesterol or blood lipids” with no language restrictions, which was updated by the applicant to February 2011 using the same search terms plus “heart disease” or “coronary heart disease”. Relevant papers were hand searched and experts in the field were contacted to identify further studies.

The search aimed to identify randomised controlled trials (RCTs) on the effects of ISP (isoflavone content >0.7 mg per g protein) on blood cholesterol concentrations in healthy, non-obese, normolipidaemic or mildly hyperlipidaemic adults which used a control food with no effect on blood cholesterol and adjusted the results at least by the subjects’ age. Exclusion criteria were enrolment of diseased or obese subjects, ISP intake >40 g per day, use of isoflavone-depleted ISP either as

intervention or as control, administration of ISP with other food constituents, “flawed” design (e.g. differences in primary outcome measures at baseline between groups, marked changes in body weight or medication use during the study, which may have confounded the results), and unbalanced energy, fat and/or fibre intakes between groups from the study foods or background diet.

The applicant identified 23 RCTs, which used ISP and one RCT, which used the water insoluble fraction of a partially hydrolysed soy protein as interventions, for the scientific substantiation of the claim, and presented an unpublished meta-analysis of 23 of these 24 RCTs. In addition, six RCTs, which used whole soy foods, and eight observational studies were provided, as well as one animal and two *in vitro* studies on the mechanism by which ISP could exert the claimed effect. Eleven published meta-analyses and one unpublished systematic review were presented in support of the claim.

The Panel notes that most of the intervention studies considered as pertinent by the applicant used animal protein (e.g. total milk protein, casein and milk) or carbohydrates as control. The Panel considers that animal protein and carbohydrates can be considered as neutral regarding their effects on blood cholesterol in humans, and consequently as an appropriate comparator to assess the effects of ISP on blood cholesterol concentrations.

The Panel considers that the study (Maki et al., 2010) which assessed the effect of the water insoluble fraction of a partially hydrolysed soy protein on blood cholesterol concentrations cannot be used to substantiate a claim on ISP owing to its compositional differences, which might have had an impact on the claimed effect.

Four of the 23 RCTs provided on ISP did not allow conclusions to be drawn on the effects of ISP on blood cholesterol concentrations owing to inadequate methodology or insufficient reporting. The statistical analysis performed for the study by Washburn et al. (1999), which had a multiple cross-over design, was insufficiently described. In the study by West et al. (2005), results were only presented as sub-group analyses according to sex and medication use, with no indication on whether these sub-group analyses had been pre-planned. The statistical analysis for another cross-over study (Blum et al., 2003) was inappropriate for the design of the study, owing to the lack of blood lipid measurements after the wash-out period and the lack of formal modelling. In the study by Hoie et al. (2005a), the rationale for undertaking multiple statistical analyses (ANOVA, MANOVA and MANCOVA) to assess the effects of treatment over time was unclear. The Panel considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

Fourteen of the remaining 19 RCTs had a parallel design while five (Evans et al., 2007; Greany et al., 2004; McVeigh et al., 2006; Steinberg et al., 2003; Thorp et al., 2008) had a cross-over design. All subjects (n=1,947) who entered data analysis had normal or moderately elevated blood LDL-cholesterol concentrations at baseline. In eight of the 19 RCTs, background diet was not assessed and possible confounding effects resulting from differences in background diet between the intervention and the control were not taken into account. Dietary intake was assessed in the remaining 11 studies, either by food frequency questionnaires (Allen et al., 2007; Crouse et al., 1999; Kreijkamp-Kaspers et al., 2004; Thorp et al., 2008) or by three-day dietary records (Baum et al., 1998; Greany et al., 2004; McVeigh et al., 2006; Santo et al., 2008; Steinberg et al., 2003; Teixeira et al., 2000; Van Horn et al., 2001).

The Panel notes that the results from the majority of these 19 studies are at high risk of bias owing to methodological limitations in the statistical analyses performed (e.g. inadequate handling or no consideration of missing data, repeated measures and/or multiple comparisons not taken into account), and that power calculations were only performed in three studies (Crouse et al., 1999; Kreijkamp-Kaspers et al., 2004; Radhakrishnan et al., 2009). The Panel also notes that group analyses, which were not pre-planned, were performed in one study (Greany et al., 2004), and that *post-hoc* analyses, despite the lack of a treatment effect assessed by analysis of variance, were conducted in two other studies (Evans et al., 2007; Hoie et al., 2007). The Panel considers that no conclusions can be drawn

from these secondary analyses for the scientific substantiation of the claim. Therefore, only primary analyses of these studies are taken into account for this assessment.

Fourteen of the 19 RCTs reported no significant effect of ISP on blood LDL (Allen et al., 2007; Evans et al., 2007; Greany et al., 2004; Hoie et al., 2007; Kreijkamp-Kaspers et al., 2004; Maesta et al., 2007; McVeigh et al., 2006; Santo et al., 2008; Steinberg et al., 2003; Teede et al., 2001; Teede et al., 2005; Thorp et al., 2008; Van Horn et al., 2001) or on non-HDL (Sagara et al., 2003) cholesterol concentrations relative to controls. These studies together considered 1,520 subjects for data analysis (9-98 subjects per group/period) and provided 20 to 40 g per day of ISP for four weeks to 12 months. The intervention period was preceded by a run-in phase in two studies (Allen et al., 2007; Van Horn et al., 2001). Intention-to-treat analyses (either “modified” or with baseline measures carried forward) were performed in two studies (Allen et al., 2007; Kreijkamp-Kaspers et al., 2004), while the remaining studies presented per protocol or completers analyses only. Four of the studies randomised, per group/period, about 100 subjects of whom around 90 were taken into account in the analyses (Allen et al., 2007; Kreijkamp-Kaspers et al., 2004; Teede et al., 2001; Thorp et al., 2008). One of these studies (Kreijkamp-Kaspers et al., 2004) was powered to detect a 7.4 % difference in total cholesterol concentrations between groups ($\beta=0.2$; $\alpha=0.05$). Two studies were small (around 10 subjects per group) and possibly underpowered to detect moderate changes in blood cholesterol concentrations (Maesta et al., 2007; Santo et al., 2008). The Panel notes that there was a general lack of information on the statistical methodology applied in the five cross-over studies (Evans et al., 2007; Greany et al., 2004; McVeigh et al., 2006; Steinberg et al., 2003; Thorp et al., 2008), which did not allow an assessment of the appropriateness of the statistical analysis performed in each of these studies.

In the RCT by Crouse et al. (1999), 151 subjects, of whom 118 (around 30 per group) entered data analysis in the relevant groups, received 25 g of ISP with varying doses of isoflavones (3 mg, 27 mg, 37 mg and 62 mg) or a control protein for nine weeks. The treatment arm where subjects consumed isoflavone-depleted (3 mg) ISP was not considered by the applicant as pertinent to the claim. The study was powered to detect a 6 % relative change in blood LDL-cholesterol concentrations between groups ($\beta=0.05$; $\alpha=0.05$). A statistically significant effect of ISP on blood LDL-cholesterol concentrations compared to control was only observed in the group receiving ISP with the highest dose of isoflavones (62 mg), while blood LDL-cholesterol concentrations did not differ significantly between the other two isoflavone-containing (27 mg and 37 mg) ISP arms and the control. The Panel notes that only one of the three relevant intervention arms which used the food which is the subject of the claim as defined by the applicant, showed an effect on LDL-cholesterol concentrations and that this study does not show a consistent effect of ISP (as defined by the applicant) on blood LDL-cholesterol concentrations.

Only four of the 19 RCTs reported a statistically significant effect of ISP on blood LDL (Hoie et al., 2005b; Radhakrishnan et al., 2009) or non-HDL (Baum et al., 1998; Teixeira et al., 2000) cholesterol concentrations. These studies considered a total of 309 subjects for data analyses (15-41 subjects per group) and provided 20 to 40 g of ISP per day for six to 24 weeks. The intervention was preceded by a run-in phase in two studies (Baum et al., 1998; Teixeira et al., 2000) and statistical analyses in all the studies were carried out per protocol or in the complete case population. One study (Radhakrishnan et al., 2009) was powered to detect a 7 % decrease in cholesterol concentrations between groups ($\beta=0.2$; $\alpha=0.05$).

The Panel notes that four RCTs with 15-41 subjects per group reported a statistically significant effect of ISP on blood LDL/non-HDL cholesterol concentrations at doses of 20 to 40 g per day consumed for six to 24 weeks. However, the Panel also notes that 14 RCTs with >15 subjects per group/period (four of which had about 90 subjects per group/period) did not report such an effect under similar conditions (20 to 40 g ISP per day given for at least six weeks in all but four studies), and that the effects found in one RCT were not consistent. In addition, most of the RCTs were at high risk of bias,

and differences in the results obtained between trials appeared to be unrelated to the dose of ISP used, sample size or study duration.

The applicant also provided a meta-analysis performed on the 24 RCTs presented as the main body of evidence for the scientific substantiation of the claim. One study was excluded from the meta-analysis as data on the mean and on the SEM or SD were not available for blood cholesterol concentrations (Radhakrishnan et al., 2009). The 23 RCTs included 32 relevant treatment arms (22 parallel and 10 cross-over design), provided 12 to 40 g of soy protein per day for 4-52 weeks and considered altogether 2,453 subjects with baseline LDL-cholesterol concentrations of 2.68-4.62 mmol/L for data analysis. Publication bias was assessed and not identified. Data were analysed using both the fixed effect and the random effects model. The Panel notes that this meta-analysis did not include any additional studies to those provided individually by the applicant for the scientific substantiation of the claim. The Panel also notes that this meta-analysis includes the four RCTs identified above as having inadequate methodology or insufficient reporting, as well as the one RCT which used the water insoluble fraction of a partially hydrolysed soy protein as intervention, and considers that this meta-analysis does not provide any additional information to the individual studies considered for the scientific substantiation of the claim.

Ten of the 11 published meta-analyses provided were not designed to assess the effects of ISP but rather of isoflavones or of soy protein from different sources, including soy foods, on blood cholesterol concentrations (Anderson et al., 1995; Anderson and Bush, 2011; Balk et al., 2005; Harland and Haffner, 2008; Hooper et al., 2008; Jenkins et al., 2010; Taku et al., 2007; Weggemans and Trautwein, 2003; Zhan and Ho, 2005; Zhuo et al., 2004). Although results of (sub-group) analyses for ISP were presented in some of the meta-analyses, all of these contained studies not considered pertinent to the claim by the applicant, or the reporting in the publication was insufficient for a full scientific evaluation. Also, the meta-analysis by Reynolds et al. (2006), which was designed to assess the effect of ISP on blood lipid concentrations, and included 27 RCTs with a total of 41 comparisons, took into account 11 studies which were considered by the applicant as not pertinent to the claim owing to methodological limitations in the design, to the high doses of ISP used, or to the characteristics of the study population. Similarly, in the unpublished systematic review (Solae, 2008, unpublished), which was performed to assess the effects of soy protein and soy isoflavones (rather than of ISP) on blood cholesterol concentrations, 49 studies were reviewed, of which only 19 were considered by the applicant as strictly pertinent to the claim. The Panel considers that no conclusions can be drawn from these meta-analyses and the systematic review for the scientific substantiation of the claim.

The six RCTs, which were provided by the applicant as supportive evidence for the substantiation of the claim, used whole soy foods as the intervention, investigated the effects of soy drinks and/or soy yoghurt (Gardner et al., 2007; Meyer et al., 2004; Takatsuka et al., 2000), a breakfast cereal produced from defatted soy flour (Jenkins et al., 2000), soy foods made of whole soy beans, soy flour and a soy drink (Matthan et al., 2007) and tofu (Ashton and Ball, 2000) on blood cholesterol concentrations. Similarly, the epidemiological studies (Ho et al., 2000; 2006; Kokubo et al., 2007; Nagata et al., 1998; Nagata et al., 2002; Rosell et al., 2004; Zhang et al., 2008; 2003) investigated the association between consumption of whole soy foods and the blood lipid profile, the risk of coronary heart disease and the risk of all-cause and cause-specific mortality. The Panel notes that the macronutrient and fibre composition of whole soy foods, which might have had an impact on the claimed effect, differs from the macronutrient composition of ISP for which the claim is made, and considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim on ISP.

With regard to a possible mechanism by which ISP could exert the claimed effect, the applicant suggests that peptides derived from the intestinal digestion of ISP can enter the circulation and exert a direct effect on the hepatic metabolism of cholesterol by increasing the expression of the hepatic LDL receptor. As evidence for this mechanism, the applicant provided two *in vitro* studies on the effect of the soybean 7S globulin α' subunit and a purified recombinant polypeptide containing the N-terminal

extension region of the soybean α' subunit on LDL-receptor mediated LDL uptake and degradation in a human hepatoma cell line (Hep G2) (Consonni et al., 2010; Lovati et al., 2000). The applicant also presented one animal study in male Sprague-Dawley rats (Duranti et al., 2004) which assessed the effect of soybean 7S globulin and soybean 7S globulin α' subunit on blood cholesterol concentrations and β -VLDL receptor activity. The Panel considers that results from rat studies cannot be extrapolated to humans because of differences in lipid metabolism between these two species, that the evidence provided did not establish that peptides derived from the intestinal digestion of ISP can be absorbed intact, and that the evidence provided in the *in vitro* studies is not sufficient to predict an effect of peptides derived from the soybean 7S globulin α' subunit on LDL-receptor mediated LDL uptake and degradation in humans.

In weighing the evidence, the Panel took into account that under similar conditions four RCTs reported an effect of ISP on blood LDL/non-HDL cholesterol concentrations, whereas 14 RCTs did not report such an effect, and another RCT showed no consistent effects. The Panel also took into account that most of these RCTs were at high risk of bias, that differences in the results obtained between trials appear unrelated to the dose of ISP used, to sample size or to study duration, and that the evidence provided in support of a possible mechanism was not convincing.

The Panel concludes that a cause and effect relationship has not been established between the consumption of ISP (as defined by the applicant) and a reduction in blood LDL-cholesterol concentrations.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, ISP (as defined by the applicant), which is the subject of the claim, is sufficiently characterised in relation to the claimed effect.
- A reduction of blood LDL-cholesterol concentrations is a beneficial physiological effect. A reduction in blood LDL-cholesterol concentrations reduces the risk of CHD.
- A cause and effect relationship has not been established between the consumption of ISP (as defined by the applicant) and a reduction in blood LDL-cholesterol concentrations.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on isolated soy protein and reduction of blood LDL-cholesterol concentrations pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0304_BE). June 2011. Submitted by ENSA/EUVEPRO/SPA.

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GLOSSARY AND ABBREVIATIONS

ANOVA	Analysis of variance
CHD	Coronary heart disease
ENSA	European Natural Soyfood Manufacturers Association
EUVEPRO	European Vegetable Protein Federation
HDL	High-density lipoprotein
ISP	Isolated soy protein
LDL	Low-density lipoprotein
MANCOVA	Multivariate analysis of covariance
MANOVA	Multivariate analysis of variance
RCT	Randomised controlled trial
SD	Standard deviation
SEM	Standard error of the mean
SPA	Soya Protein Association